I. AZLACTONES
II. METABOLISM OF FATTY ACIDS

BY

CARL MANTLE STEVENS

B. A., American University, 1937

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY
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PART I
AZLACTONES

Introduction

The use of azlactones as intermediates in standard methods for the preparation of amino acids and dipeptides, makes their study of considerable biochemical interest. The present work is an investigation of the chemistry of azlactones with particular reference to their role in the racemization of amino acids by acetic anhydride.

Experimental

The racemization of optically active acylamino acids in acetic anhydride was investigated. Benzoyl-*dl*-*p*-methoxyphenylalanine azlactone was obtained in 22 per cent yield in the racemization of benzoyl-*l*-*p*-methoxyphenylalanine by one mole of acetic anhydride. Addition of larger quantities of acetic anhydride resulted in more rapid racemization, and a greater yield of azlactone.

Azlactones react with acetic acid to yield acetic anhydride. Azlactones cause a very rapid racemization of optically active acylamino acids. The rate of racemization by azlactone is much greater than that by an equivalent amount of acetic anhydride. This indicates that there is a direct reaction between azlactone and acylamino acid. This reaction must be reversible since one mole of azlactone will racemize several moles of optically active acylamino acid.

The racemization of optically active acylamino acids in aqueous acetic acid was investigated. Pure benzoyl-*dl*-*p*-methoxyphenylalanine azlactone was isolated in 42 per cent yield in the racemization of benzoyl-*l*-*p*-methoxyphenylalanine by the procedure of du Vigneaud and Meyer.[1]

Sodium acetate is known to accelerate azlactone formation.[2] It accelerates the rate of racemization of acylamino acids in glacial acetic acid by acetic anhydride, but does not affect

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1. du Vigneaud, V., and Meyer, C. E., J. Biol. Chem., **99**, 143 (1932-33)
2. Carter, H. E., and Stevens, C. M., J. Biol. Chem., **133**, 117 (1940)

racemization by preformed azlactone. Therefore, the principal effect of sodium acetate on racemization in glacial acetic acid is to accelerate azlactone formation.

Benzoyl-*d*-N-methylphenylalanine in glacial acetic acid is rapidly racemized by acetic anhydride, while *p*-toluenesulfonyl-*l*-leucine is not.

Benzyl mercaptan adds to benzoyl-α-aminocrotonic acid azlactone I to give an 80 per cent yield of the isomeric benzoyl-α-amino-β-benzylthio-*n*-butyric acids. These were separated by conversion to the β-phenethylamine salts. The isomeric benzoyl-α-amino-β-benzylthio-*n*-butyric acids were hydrolyzed to the α-amino-β-benzylthio--*n*-butyric acids which were reduced with sodium in liquid ammonia to the α-amino-β-thiol-*n*-butyric acids. The thiolamino acids give the characteristic reactions of cysteine, including the highly specific Sullivan test.[3]

Summary

Formation of azlactones, reaction between azlactones and acylamino acids, and reversibility of azlactonization provide an adequate explanation of "catalytic" racemization of acylamino acids by acetic anhydride in glacial acetic acid.[4] Sodium acetate accelerates the racemization principally by accelerating azlactone formation.

The α-amino-β-thiol-*n*-butyric acids have been synthesized.

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3. Sullivan, M. X., U. S. Public Health Reports, **44**, 1421 (1929)
4. Bergmann, M., and Zervas, L., Biochem. Z., **203**, 280 (1928)

PART II

METABOLISM OF FATTY ACIDS

Introduction

It is generally believed that fatty acids are metabolized in the animal body by successive oxidations at the β-carbon atom. By feeding large doses of certain fats, Verkade and coworkers[1] have demonstrated an oxidation of fatty acids at the terminal methyl group (ω-oxidation) to yield the corresponding dibasic acids. Dibasic acids were recovered in the urine only after the feeding of fats containing acids of 8 to 11 carbon atoms. The present work was designed to study the factors affecting ω-oxidation in compounds where β-oxidation was impossible.

Experimental

The compounds investigated were β-phenyl fatty acids having chain lengths of from 6 to 12 carbon atoms. The free acids were fed to dogs on a normal stock diet.

β-Phenylhexanoic, β-phenylheptanoic, and β-phenyloctanoic acids were recovered from the urine unchanged in yields of 75, 50, and 40 per cent, respectively. With longer chains, only a very small amount of the unchanged acid was excreted. Instead, oxidation products were recovered amounting to approximately 20 per cent of the amount fed. From the feeding of β-phenyldecanoic and β-phenyldodecanoic acids, yields of 6.5 and 7.7 per cent, respectively, of pure β-phenyladipic acid were isolated. It is highly probable that this compound was formed by ω-oxidation followed by two or three subsequent β-oxidations.

Summary

A striking effect of chain length on ω-oxidation of fatty acids has been demonstrated by feeding a series of β-phenyl fatty acids to dogs. Only the acids having chain lengths of 8 carbon atoms or less are excreted unchanged. The acids of longer chain length are excreted in part at least as dibasic acids formed by ω-oxidation with subsequent shortening of the chain by β-oxidation.

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1. Verkade, P. E., van der Lee, J., Biochem. J., 28, 31 (1934)

VITA

The author was born in Washington, D. C., October 31, 1915. He graduated from Washington-Lee High School, Arlington, Virginia, in June, 1933, and entered American University the following September. In June, 1937, he was granted the degree of Bachelor of Arts by American University. The following year, he was employed in the laboratory of the International Paper Company, Livermore Falls, Maine. He entered the Graduate School of the University of Illinois in February, 1938. From February, 1939, to June, 1939, he was assistant in the Department of Chemistry. Since September, 1939, he has held a University Fellowship.

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